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(54) **Production process of kefir-like fermented milk.**

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16, April 1986, pages 483-490, Munich, DE; H.  
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**PATENT ABSTRACTS OF JAPAN**, vol. 13, no.  
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## D scription

### 1) Field of the Invention:

This invention relates to a process for producing kefir or a similar fermented milk, which contains lactic acid and ethanol.

### 2) Description of the Related Art:

Kefir, a sort of fermented milk, is produced by simultaneously conducting lactic acid fermentation with a lactic acid bacterium and alcoholic fermentation with a yeast. Unlike ordinary fermented milks produced by using a lactic acid bacterium alone, kefir contains both ethanol and carbon dioxide gas and has characteristic flavor and taste. It is traditional popular fermented milk habitually taken in the Eastern Europe and Russia. In recent years, it is also sold in Japan although the consumption is still at a low level.

A process for the preparation of a fermented milk from a raw material which is fermented without addition of any sugar by using in combination (a) a lactic acid bacterium capable of producing glucose from lactose and (b) a lactose non-fermenting yeast, capable of coexisting with the lactic acid bacterium (a) is described in FR-A-2123295. The process intends to impart an aromatic flavor to the resulting fermented milk.

Kefir is generally produced by using mixed cell aggregates called "kefir grains". Kefir grains contain plural lactic acid bacteria and plural yeasts. Using as a binder viscous polysaccharides produced externally by the lactic acid bacteria, the individual cells are bound together to have a granular form. Exemplary lactic acid bacteria in kefir grains include streptococci such as *Streptococcus lactis*, *Streptococcus cremoris* and *leuconostocs* as well as lactobacilli. Many yeasts have been recognized, including *Candida pseudotropicalis*, *Candida kefir*, *Saccharomyces lactis*, *Saccharomyces fragilis*, *Saccharomyces unisporus*, *Saccharomyces bulgaricus*, *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* (see Deutsche Molkezeitung, Vol. 107 (1986) pages 483 - 490).

When kefir is produced using kefir grains, the lactic acid bacteria and yeasts contained in the kefir grains remain in the product as they are. The lactic acid fermentation practically stops as the pH drops, but the alcoholic fermentation may still proceed by some yeasts capable of assimilating as sugar sources lactose and other sugars still remaining after packing of the product in containers provided that temperature conditions are met. Upon marketing of kefirs produced by the conventional processes, they are accompanied by the drawback that the secondary alcoholic fermentation tends to

occur at the distribution stage of the products, thereby resulting not only in substantial changes in flavor and taste due to the formation of ethanol and carbon dioxide gas but also in bulging of containers and leakage of the contents due to the internal pressure increased by carbon dioxide gas.

On the other hand, it has been desired to develop kefir-like fermented milk products which may allow kids to conveniently enjoy the characteristic flavor and taste of kefir. For this purpose, it has been necessary to control the formation of ethanol as much as possible throughout the fermentation and production steps.

## SUMMARY OF THE INVENTION

With the foregoing in view, the present inventors have conducted an extensive investigation with a view toward developing a process for the production of kefir or a kefir-like fermented milk, which allows to control the formation of ethanol. As a result, the formation of ethanol has been found controllable as desired when the alcoholic fermentation is conducted with a yeast which assimilates neither lactose remaining in the product nor galactose to be formed by lactic acid fermentation, leading to completion of this invention.

The present invention provides a process for the production of a kefir-like fermented milk from a raw material containing lactose and at least one sugar, including subjecting the lactose to lactic acid fermentation with a lactic acid bacterium and the sugar to alcoholic fermentation with a yeast, characterized in that a sugar selected from glucose, fructose, sucrose and mixtures thereof is added to a milk and the alcoholic fermentation is conducted with a lactose non-fermenting and galactose non-fermenting yeast until all assimilable sugar sources are substantially eliminated.

The conventional production processes of kefir require kefir grains which contain various lactic acid bacteria and yeasts. Therefore, it has heretofore been impossible to prevent secondary alcoholic fermentation of remaining lactose, galactose and the like as sugar sources at the distribution stage of the products. It has also been difficult to control the alcoholic fermentation even in the course of the production, leading to the drawback that the flavor and taste can hardly be stabilized. The process of the present invention allows the alcoholic fermentation to proceed under control in the course of the production, thereby completely solving the problem of subsequent alcoholic fermentation. The production process of this invention has therefore made it possible to easily produce a kefir-like fermented milk having stable quality and excellent storability.

The above and other objects, features and advantages of the present invention will become ap-

parent from the following description and the appended claims.

#### DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

The term "kefir-like fermented milk or milk products" as used herein means not only kefir itself but also various similar fermented milk products. Namely, exemplary kefir-like fermented milk products are led by the above-described kefir-like fermented milk products containing no ethanol or having an extremely low ethanol content and include all fermented milk products somewhat different from kefir in milk solid contents, flavor, taste, flowability, etc. so long as they are produced by lactic acid fermentation and alcoholic fermentation.

In lactic acid fermentation, as is known well, a lactic acid bacterium induces fermentation of lactose contained in milk to produce lactic acid. Here, many lactic acid bacteria do not consume much of galactose which is one of the constituent sugars of lactose, so that galactose is accumulated either partly or wholly in the culture broth. On the other hand, the yeasts contained in kefir grains are classified into lactose fermenting ones and lactose non-fermenting ones. Both of these two types of yeasts however have galactose fermenting ability. Sugars which are fermented by the yeasts employed in conventional kefir production are lactose and galactose formed by lactic acid fermentation.

In contrast, the process of the present invention requires the use of a lactose non-fermenting and galactose non-fermenting yeast, which is capable of fermenting neither lactose nor galactose, so as to control alcoholic fermentation.

Such yeasts have already been known and are available. Typical examples of these yeasts are as follows:

##### (1) Sucrose non-fermenting yeasts:

Saccharomyces bisporus IFO-0467, Saccharomyces mellis, Saccharomyces rouxii, Kuloekera africana, Kuloekera apiculata, Torulopsis atomiae, and Torulopsis castellii.

##### (1) Sucrose fermenting yeasts:

Saccharomyces bayanus, Saccharomyces fermentati, Candida solani IFO-0762, Kuloekera javanica, Kuloekera jensenii, Torulopsis bacillaris, and Torulopsis colliculosa, IFO-0381.

In the process of this invention, mammal's milk is usually employed as one of main raw materials. It is possible to use either an economically-available fresh milk such as cow's milk, goat's milk, horse's milk or buffalo's milk or a milk reconstituted

from skim milk or powder milk.

The lactic acid fermentation can be conducted using a lactic acid bacterium employed usually for the production of a fermented milk or any other desired lactic acid bacterium. The fermentation does not require any special conditions.

The alcoholic fermentation is effected using a lactose non-fermenting and galactose non-fermenting yeast such as that referred to above. As the sugar to be consumed as a raw material in the alcoholic fermentation, a sugar is added separately without depending wholly on one or more sugar components of mammal's milk. The sugar is generally added in an amount necessary to form, by the alcoholic fermentation, ethanol and carbon dioxide in amounts desired to be contained in the final product. It is not desirable to use the sugar in any amounts greater than that level. The amount of the sugar to be added can be calculated. It can also be determined easily by an experiment.

The lactic acid fermentation and alcoholic fermentation may be conducted at the same time, or the lactic acid fermentation may be effected first, followed by the alcoholic fermentation.

For simultaneous fermentation, a milk containing a desired amount of a sugar for alcoholic fermentation added thereto is inoculated with starters of both lactic acid bacterium and yeast and is then incubated at a suitable temperature for fermentation.

For sequential fermentation, upon substantial completion of the lactic acid fermentation by a method known per se in the art, the culture broth is inoculated with a yeast starter to conduct the alcoholic fermentation. In this case, the sugar to be consumed in the alcoholic fermentation may desirably be added upon inoculation with the yeast starter. It is also possible to add the sugar to the mammal's milk before starting the lactic acid fermentation. (Even if the sugar is fermentable by the lactic acid bacterium, the lactic acid bacterium primarily consumes lactose in the milk and the amount of the sugar required for the alcoholic fermentation is not substantially different from a sugar unfermentable by the lactic acid bacterium.) As an alternative, upon inoculation of the milk with the lactic acid bacterium starter, the milk may also be inoculated with the yeast starter without addition of a sugar source for alcoholic fermentation. After completion of the lactic acid fermentation, the sugar source for alcoholic fermentation is added to initiate the growth of the yeast.

As the sugar to be added for the alcoholic fermentation, it is possible to use glucose, fructose or sucrose or a mixture therefore, e.g., isomerized sugar, fruit juice or the like.

Whichever fermentation method is employed, the alcoholic fermentation is allowed to proceed

until the thus-added sugar for the alcoholic fermentation is substantially eliminated, preferably, until the content of the sugar thus added becomes 0.01% or less in the culture broth. The production process is thus completed.

After completion of the fermentations in the above manner without remainder of any substantial portion of the sugar source for the alcoholic fermentation, post processing is applied as needed depending on the kind of the product.

When it is desired to add a sweetening and/or a fruit juice to the product, sugars contained in the sweetening and/or fruit juice must not be fermentable by the yeast employed for the alcoholic fermentation and still remaining in order to avoid any secondary alcoholic fermentation. Where the yeast is sucrose fermenting for example, sucrose must not be used as a sweetening. Where the yeast is lactose non-fermenting and galactose non-fermenting and also sucrose non-fermenting, for example, Saccharomyces bisporus IFO-0467, it is possible to use as a sweetening sucrose whose sweetness characteristics and the like are best.

#### [Examples]

The present invention will hereinafter be described in further detail by the following examples. It should however be borne in mind that this invention is not limited to the following examples.

#### Example 1:

Reconstituted skim milk having a nonfat milk solids content of 18% w/v was inoculated with 0.5% of a starter of Streptococcus thermophilus - (IFO-3535), followed by culture at 37°C for 20 hours. When the pH had dropped to 4.50, the culture broth was cooled. Mixed with 500 parts of the culture broth were 500 parts of an aqueous solution which contained sugars as sweetening and sugar sources for alcohol fermentation, a fruit juice, a stabilizer (high methoxyl pectin), etc. Of the sugars added, the amount of the sugars fermentable by Saccharomyces bisporus, namely, glucose and fructose was 6 parts.

The resultant mixture was inoculated with 1.0% of a starter of Saccharomyces bisporus (IFO-0467) which had been prepared separately. Thereafter, the mixture was incubated at 30°C for 18 hours. After confirming that the total content of glucose and fructose had been reduced to 0.01% or lower in the culture broth, the culture broth was cooled to 5°C or lower, homogenized and then packed in sealed containers.

The kefir-like fermented milk obtained as described above contained 0.3% of ethanol which had been produced by the yeast. It had char-

acteristic flavor and taste attributable to the ethanol and carbon dioxide and was delicious. Even when the product was left over at room temperature, evolution of carbon dioxide by secondary alcoholic fermentation was practically unobserved so that the product retained its original flavor and taste for a long time.

#### Example 2:

Reconstituted skim milk having a nonfat milk solids content of 18% w/v was inoculated with 0.5% of a starter of Streptococcus thermophilus - (IFO-3535) and 1.0% of a starter of Candida solani (IFO-0762), followed by culture at 37°C for 20 hours. When the pH had dropped to 4.50, the culture broth was cooled. Mixed with 500 parts of the culture broth were 500 parts of an aqueous solution which contained sugars as sweetening and sugar sources for alcohol fermentation, a fruit juice, a stabilizer (high methoxyl pectin), etc. Of the sugars added, the amount of the sugars fermentable by Candida solani, namely, sucrose, glucose and fructose was 6 parts. The resultant mixture was then incubated at 30°C for 18 hours. After confirming that the total content of sucrose, glucose and fructose had been reduced to 0.01% or lower in the culture broth, the culture broth was cooled to 5°C or lower, homogenized and then packed in sealed containers.

The kefir-like fermented milk obtained as described above contained 0.3% of ethanol which had been produced by the yeast. It had characteristic flavor and taste attributable to the ethanol and carbon dioxide and was delicious. Even when the product was left over at room temperature, evolution of carbon dioxide by secondary alcoholic fermentation was practically unobserved so that the product retained its original flavor and taste for a long time.

#### Example 3:

Reconstituted skim milk having a nonfat milk solids content of 18% w/v and containing 1.2% of sucrose as a sugar source for alcoholic fermentation was inoculated with 0.5% of a starter of Streptococcus thermophilus (IFO-3535) and 1.0% of a starter of Torulopsis colliculosa (IFO-0381), followed by culture at 34°C for 24 hours. After confirming that the pH had dropped to 4.1-4.2 and the total content of sucrose, glucose and fructose had been reduced to 0.01% or lower in the culture broth, the culture broth was cooled. Mixed with 500 parts of the culture broth were 500 parts of an aqueous solution which contained a sweetening (Aspartame) and a stabilizer (high methoxyl pectin), etc. The resultant mixture was then cooled to 5°C

or lower, homogenized and then packed in sealed containers.

The kefir-like fermented milk obtained as described above contained 0.3% of ethanol which had been produced by the yeast. It had characteristic flavor and taste attributable to the ethanol and carbon dioxide and was delicious. Even when the product was left over at room temperature, evolution of carbon dioxide by secondary alcoholic fermentation was practically unobserved so that the product retained its original flavor and taste for a long time.

#### Claims

1. A process for the production of a kefir-like fermented milk from a raw material containing lactose and at least one sugar, including subjecting the lactose to lactic acid fermentation with a lactic acid bacterium and the sugar to alcoholic fermentation with a yeast, characterized in that a sugar selected from glucose, fructose, sucrose and mixtures thereof is added to a milk and the alcoholic fermentation is conducted with a lactose non-fermenting and galactose non-fermenting yeast until all assimilable sugar sources are substantially eliminated.
2. The process as claimed in Claim 1, wherein the lactic acid fermentation and alcoholic fermentation are conducted simultaneously.
3. The process as claimed in Claim 1, wherein the lactic acid fermentation and alcoholic fermentation are conducted sequentially.
4. The process as claimed in Claim 1, wherein the lactose non-fermenting and galactose non-fermenting yeast is also sucrose non-fermenting.
5. The process as claimed in Claim 4, further comprising adding sucrose as a sweetening.
6. The process as claimed in Claim 1, wherein the raw material contains mammal's milk.
7. The process as claimed in Claim 6, wherein the mammal's milk is selected from cow's milk, goat's milk, horse's milk or buffalo's milk.

#### Patentansprüche

1. Verfahren zum Herstellen einer kefirartigen gegorenen Milch aus einem Rohmaterial, das Lactose und mindestens einen Zucker enthält, durch Milchsäuregärung der Lactose mit ei-

nem Milchsäurebakterium und alkoholische Gärung des Zuckers mit einer Hefe, dadurch gekennzeichnet, daß ein Zucker, ausgewählt aus Glucose, Fructose, Saccharose und deren Mischungen, zu einer Milch hinzugegeben wird und die alkoholische Gärung mit einer Lactose nicht-vergärenden und Galactose nicht-vergärenden Hefe ausgeführt wird, bis alle assimilierbaren Zuckerquellen im wesentlichen beseitigt sind.

2. Verfahren nach Anspruch 1, worin die Milchsäuregärung und die alkoholische Gärung gleichzeitig ausgeführt werden.
3. Verfahren nach Anspruch 1, worin die Milchsäuregärung und die alkoholische Gärung nacheinander ausgeführt werden.
4. Verfahren nach Anspruch 1, worin die Lactose nicht-vergärende und Galactose nicht-vergärende Hefe auch Saccharose nicht vergärt.
5. Verfahren nach Anspruch 4, weiter umfassend die Zugabe von Saccharose als einem Süßmittel.
6. Verfahren nach Anspruch 1, worin das Rohmaterial Säugetiermilch enthält.
7. Verfahren nach Anspruch 6, worin die Säugetiermilch ausgewählt ist aus Kuhmilch, Ziegenmilch, Stutenmilch oder Büffelmilch.

#### Revendications

1. Procédé pour produire un lait fermenté de type kéfir à partir d'une matière première contenant du lactose et au moins un sucre, qui comprend l'opération consistant à soumettre le lactose à une fermentation lactique avec un ferment lactique et le sucre à une fermentation alcoolique avec une levure, caractérisé en ce qu'un sucre choisi parmi le glucose, le fructose, le saccharose et leurs mélanges est ajouté à un lait, et que la fermentation alcoolique est mise en oeuvre avec une levure ne fermentant ni le lactose, ni le galactose, jusqu'à élimination pratiquement complète de la totalité des sources de sucre assimilables.
2. Procédé selon la revendication 1, dans lequel la fermentation lactique et la fermentation alcoolique sont mises en oeuvre simultanément.
3. Procédé selon la revendication 1, dans lequel la fermentation lactique et la fermentation alcoolique sont mises en oeuvre successive-

ment.

4. Procédé selon la revendication 1, dans lequel la levure qui ne fermente pas le lactose et ne fermente pas le galactose ne fermente pas non plus le saccharose. 5
5. Procédé selon la revendication 4, qui comprend, en outre, l'addition de saccharose en tant qu'édulcorant. 10
6. Procédé selon la revendication 1, dans lequel la matière première contient du lait de mammifère. 15
7. Procédé selon la revendication 6, dans lequel le lait de mammifère est choisi parmi le lait de vache, le lait de chèvre, le lait de jument et le lait de bufflesse. 20

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⑦④ Representative: **Wächtershäuser, Günter, Dr.**  
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⑤④ Production process of kefir-like fermented milk.

⑤⑦ A kefir-like fermented milk is produced from a  
raw material, which contains lactose and at least one  
sugar, by subjecting the lactose to lactic acid fer-  
mentation with a lactic acid bacterium and the sugar  
to alcoholic fermentation with a yeast. The alcoholic  
fermentation is conducted with a lactose non-fermen-  
ting and galactose non-fermenting yeast until all  
assimilable sugar sources are substantially elimi-  
nated.

**EP 0 346 884 A2**

## PRODUCTION PROCESS OF KEFIR-LIKE FERMENTED MILK

### FIELD OF THE INVENTION

#### 1) Field of the Invention:

This invention relates to a process for producing kefir or a similar fermented milk, which contains lactic acid and ethanol.

#### 2) Description of the Related Art:

Kefir, a sort of fermented milk, is produced by simultaneously conducting lactic acid fermentation with a lactic acid bacterium and alcoholic fermentation with a yeast. Unlike ordinary fermented milks produced by using a lactic acid bacterium alone, kefir contains both ethanol and carbon dioxide gas and has characteristic flavor and taste. It is traditional popular fermented milk habitually taken in the Eastern Europe and Russia. In recent years, it is also sold in Japan although the consumption is still at a low level.

Kefir is generally produced by using mixed cell aggregates called "kefir grains". Kefir grains contain plural lactic acid bacteria and plural yeasts. Using as a binder viscous polysaccharides produced externally by the lactic acid bacteria, the individual cells are bound together to have a granular form. Exemplary lactic acid bacteria in kefir grains include streptococci such as Streptococcus lactis, Streptococcus cremoris and leuconostocs as well as lactobacilli. Many yeasts have been recognized, including Candida pseudotropicalis, Candida kefir, Saccharomyces lactis, Saccharomyces fragilis, Saccharomyces unisporus, Saccharomyces bulgaricus, Saccharomyces cerevisiae and Saccharomyces carlsbergensis.

When kefir is produced using kefir grains, the lactic acid bacteria and yeasts contained in the kefir grains remain in the product as they are. The lactic acid fermentation practically stops as the pH drops, but the alcoholic fermentation may still proceed by some yeasts capable of assimilating as sugar sources lactose and other sugars still remaining after packing of the product in containers provided that temperature conditions are met. Upon marketing of kefirs produced by the conventional processes, they are accompanied by the drawback that the secondary alcoholic fermentation tends to occur at the distribution stage of the products, thereby resulting not only in substantial changes in flavor and taste due to the formation of ethanol and carbon dioxide gas but also in bulging of contain-

ers and leakage of the contents due to the internal pressure increased by carbon dioxide gas.

On the other hand, it has been desired to develop kefir-like fermented milk products which may allow kids to conveniently enjoy the characteristic flavor and taste of kefir. For this purpose, it has been necessary to control the formation of ethanol as much as possible throughout the fermentation and production steps.

### SUMMARY OF THE INVENTION

With the foregoing in view, the present inventors have conducted an extensive investigation with a view toward developing a process for the production of kefir or a kefir-like fermented milk, which allows to control the formation of ethanol. As a result, the formation of ethanol has been found controllable as desired when the alcoholic fermentation is conducted with a yeast which assimilates neither lactose remaining in the product nor galactose to be formed by lactic acid fermentation, leading to completion of this invention.

In one aspect of this invention, there is thus provided a process for the production of a kefir-like fermented milk from a raw material containing lactose and at least one sugar, including subjecting the lactose to lactic acid fermentation with a lactic acid bacterium and the sugar to alcoholic fermentation with a yeast. The alcoholic fermentation is conducted with a lactose non-fermenting and galactose non-fermenting yeast until all assimilable sugar sources are substantially eliminated.

The conventional production processes of kefir require kefir grains which contain various lactic acid bacteria and yeasts. Therefore, it has heretofore been impossible to prevent secondary alcoholic fermentation of remaining lactose, galactose and the like as sugar sources at the distribution stage of the products. It has also been difficult to control the alcoholic fermentation even in the course of the production, leading to the drawback that the flavor and taste can hardly be stabilized. The process of the present invention allows the alcoholic fermentation to proceed under control in the course of the production, thereby completely solving the problem of subsequent alcoholic fermentation. The production process of this invention has therefore made it possible to easily produce a kefir-like fermented milk having stable quality and excellent storability.

The above and other objects, features and advantages of the present invention will become apparent from the following description and the ap-



pending claims.

# DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

The term "kefir-like fermented milk or milk products" as used herein means not only kefir itself but also various similar fermented milk products. Namely, exemplary kefir-like fermented milk products are led by the above-described kefir-like fermented milk products containing no ethanol or having an extremely low ethanol content and include all fermented milk products somewhat different from kefir in milk solid contents, flavor, taste, flowability, etc. so long as they are produced by lactic acid fermentation and alcoholic fermentation.

In lactic acid fermentation, as is known well, a lactic acid bacterium induces fermentation of lactose contained in milk to produce lactic acid. Here, many lactic acid bacteria do not consume much of galactose which is one of the constituent sugars of lactose, so that galactose is accumulated either partly or wholly in the culture broth. On the other hand, the yeasts contained in kefir grains are classified into lactose fermenting ones and lactose non-fermenting ones. Both of these two types of yeasts however have galactose fermenting ability. Sugars which are fermented by the yeasts employed in conventional kefir production are lactose and galactose formed by lactic acid fermentation.

In contrast, the process of the present invention requires the use of a lactose non-fermenting and galactose non-fermenting yeast, which is capable of fermenting neither lactose nor galactose, so as to control alcoholic fermentation.

Such yeasts have already been known and are available. Typical examples of these yeasts are as follows:

## (1) Sucrose non-fermenting yeasts:

Saccharomyces bisporus IFO-0467, Saccharomyces mellis, Saccharomyces rouxii, Kuloekera africana, Kuloekera apiculata, Torulopsis atomiae, and Torulopsis castellii.

## (1) Sucrose fermenting yeasts:

Saccharomyces bayanus, Saccharomyces fermentati, Candida solani IFO-0762, Kuloekera javanica, Kuloekera jensenii, Torulopsis bacillaris, and Torulopsis colliculosa, IFO-0381.

In the process of this invention, mammal's milk is usually employed as one of main raw materials.

It is possible to use either an economically-available fresh milk such as cow's milk, goat's milk, horse's milk or buffalo's milk or a milk reconstituted from skim milk or powder milk.

The lactic acid fermentation can be conducted using a lactic acid bacterium employed usually for the production of a fermented milk or any other desired lactic acid bacterium. The fermentation does not require any special conditions.

The alcoholic fermentation is effected using a lactose non-fermenting and galactose non-fermenting yeast such as that referred to above. As the sugar to be consumed as a raw material in the alcoholic fermentation, it is preferable to add a sugar separately without depending wholly on one or more sugar components of mammal's milk. The sugar is generally added in an amount necessary to form, by the alcoholic fermentation, ethanol and carbon dioxide in amounts desired to be contained in the final product. It is not desirable to use the sugar in any amounts greater than that level. The amount of the sugar to be added can be calculated. It can also be determined easily by an experiment.

The lactic acid fermentation and alcoholic fermentation may be conducted at the same time, or the lactic acid fermentation may be effected first, followed by the alcoholic fermentation.

For simultaneous fermentation, a milk containing a desired amount of a sugar for alcoholic fermentation added thereto is inoculated with starters of both lactic acid bacterium and yeast and is then incubated at a suitable temperature for fermentation.

For sequential fermentation, upon substantial completion of the lactic acid fermentation by a method known per se in the art, the culture broth is inoculated with a yeast starter to conduct the alcoholic fermentation. In this case, the sugar to be consumed in the alcoholic fermentation may desirably be added upon inoculation with the yeast starter. It is also possible to add the sugar to the mammal's milk before starting the lactic acid fermentation. (Even if the sugar is fermentable by the lactic acid bacterium, the lactic acid bacterium primarily consumes lactose in the milk and the amount of the sugar required for the alcoholic fermentation is not substantially different from a sugar unfermentable by the lactic acid bacterium.) As an alternative, upon inoculation of the milk with the lactic acid bacterium starter, the milk may also be inoculated with the yeast starter without addition of a sugar source for alcoholic fermentation. After completion of the lactic acid fermentation, the sugar source for alcoholic fermentation is added to initiate the growth of the yeast.

As the sugar to be added for the alcoholic fermentation, it is possible to use, for example,

glucose, fructose or sucrose or a mixture therefore, e.g., isomerized sugar, fruit juice or the like.

Whichever fermentation method is employed, the alcoholic fermentation is allowed to proceed until the thus-added sugar for the alcoholic fermentation is substantially eliminated, preferably, until the content of the sugar thus added becomes 0.01% or less in the culture broth. The production process is thus completed.

After completion of the fermentations in the above manner without remainder of any substantial portion of the sugar source for the alcoholic fermentation, post processing is applied as needed depending on the kind of the product.

When it is desired to add a sweetening and/or a fruit juice to the product, sugars contained in the sweetening and/or fruit juice must not be fermentable by the yeast employed for the alcoholic fermentation and still remaining in order to avoid any secondary alcoholic fermentation. Where the yeast is sucrose fermenting for example, sucrose must not be used as a sweetening. Where the yeast is lactose non-fermenting and galactose non-fermenting and also sucrose non-fermenting, for example, Saccharomyces bisporus IFO-0467, it is possible to use as a sweetening sucrose whose sweetness characteristics and the like are best.

#### [Examples]

The present invention will hereinafter be described in further detail by the following examples. It should however be borne in mind that this invention is not limited to the following examples.

#### Example 1:

Reconstituted skim milk having a nonfat milk solids content of 18% w/v was inoculated with 0.5% of a starter of Streptococcus thermophilus - (IFO-3535), followed by culture at 37°C for 20 hours. When the pH had dropped to 4.50, the culture broth was cooled. Mixed with 500 parts of the culture broth were 500 parts of an aqueous solution which contained sugars as sweetening and sugar sources for alcohol fermentation, a fruit juice, a stabilizer (high methoxyl pectin), etc. Of the sugars added, the amount of the sugars fermentable by Saccharomyces bisporus, namely, glucose and fructose was 6 parts.

The resultant mixture was inoculated with 1.0% of a starter of Saccharomyces bisporus (IFO-0467) which had been prepared separately. Thereafter, the mixture was incubated at 30°C for 18 hours. After confirming that the total content of glucose and fructose had been reduced to 0.01% or lower

in the culture broth, the culture broth was cooled to 5°C or lower, homogenized and then packed in sealed containers.

The kefir-like fermented milk obtained as described above contained 0.3% of ethanol which had been produced by the yeast. It had characteristic flavor and taste attributable to the ethanol and carbon dioxide and was delicious. Even when the product was left over at room temperature, evolution of carbon dioxide by secondary alcoholic fermentation was practically unobserved so that the product retained its original flavor and taste for a long time.

#### Example 2:

Reconstituted skim milk having a nonfat milk solids content of 18% w/v was inoculated with 0.5% of a starter of Streptococcus thermophilus - (IFO-3535) and 1.0% of a starter of Candida solani (IFO-0762), followed by culture at 37°C for 20 hours. When the pH had dropped to 4.50, the culture broth was cooled. Mixed with 500 parts of the culture broth were 500 parts of an aqueous solution which contained sugars as sweetening and sugar sources for alcohol fermentation, a fruit juice, a stabilizer (high methoxyl pectin), etc. Of the sugars added, the amount of the sugars fermentable by Candida solani, namely, sucrose, glucose and fructose was 6 parts. The resultant mixture was then incubated at 30°C for 18 hours. After confirming that the total content of sucrose, glucose and fructose had been reduced to 0.01% or lower in the culture broth, the culture broth was cooled to 5°C or lower, homogenized and then packed in sealed containers.

The kefir-like fermented milk obtained as described above contained 0.3% of ethanol which had been produced by the yeast. It had characteristic flavor and taste attributable to the ethanol and carbon dioxide and was delicious. Even when the product was left over at room temperature, evolution of carbon dioxide by secondary alcoholic fermentation was practically unobserved so that the product retained its original flavor and taste for a long time.

#### Example 3:

Reconstituted skim milk having a nonfat milk solids content of 18% w/v and containing 1.2% of sucrose as a sugar source for alcoholic fermentation was inoculated with 0.5% of a starter of Streptococcus thermophilus (IFO-3535) and 1.0% of a starter of Torulopsis colliculosa (IFO-0381), followed by culture at 34°C for 24 hours. After con-

firming that the pH had       ed to 4.1-4.2 and the total content of sucrose, glucose and fructose had been reduced to 0.01% or lower in the culture broth, the culture broth was cooled. Mixed with 500 parts of the culture broth were 500 parts of an aqueous solution which contained a sweetening (Aspartame) and a stabilizer (high methoxyl pectin), etc. The resultant mixture was then cooled to 5 °C or lower, homogenized and then packed in sealed containers.

The kefir-like fermented milk obtained as described above contained 0.3% of ethanol which had been produced by the yeast. It had characteristic flavor and taste attributable to the ethanol and carbon dioxide and was delicious. Even when the product was left over at room temperature, evolution of carbon dioxide by secondary alcoholic fermentation was practically unobserved so that the product retained its original flavor and taste for a long time.

### Claims

1. A process for the production of a kefir-like fermented milk from a raw material containing lactose and at least one sugar, including subjecting the lactose to lactic acid fermentation with a lactic acid bacterium and the sugar to alcoholic fermentation with a yeast, which comprises conducting the alcoholic fermentation with a lactose non-fermenting and galactose non-fermenting yeast until all assimilable sugar sources are substantially eliminated.

2. The process as claimed in Claim 1, wherein the lactic acid fermentation and alcoholic fermentation are conducted simultaneously.

3. The process as claimed in Claim 1, wherein the lactic acid fermentation and alcoholic fermentation are conducted sequentially.

4. The process as claimed in Claim 1, wherein the lactose non-fermenting and galactose non-fermenting yeast is also sucrose non-fermenting.

5. The process as claimed in Claim 4, further comprising adding sucrose as a sweetening.

6. The process as claimed in Claim 1, wherein the raw material contains mammal's milk.

7. The process as claimed in Claim 6, wherein the mammal's milk is selected from cow's milk, goat's milk, horse's milk or buffalo's milk.

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19



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54

**Production process of kefir-like fermented milk.**

57

A kefir-like fermented milk is produced from a raw material, which contains lactose and at least one sugar, by subjecting the lactose to lactic acid fermentation with a lactic acid bacterium and the sugar to alcoholic fermentation with a yeast. The alcoholic

fermentation is conducted with a lactose non-fermenting and galactose non-fermenting yeast until all assimilable sugar sources are substantially eliminated.

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	FR-A-2 123 295 (CALPIS SHOKUJIN KOGYO K.K.) * Claims 1,4,6; page 4; page 5, table 5 * ---	1,2,6,7	A 23 C 9/127
X	US-A-3 563 760 (SEJI KUWABARA) * Claims 1,2,3,8; example 1 * ---	1,3,6,7	
X	DEUTSCHE MOLKEREI-ZEITUNG, vol. 107, no. 16, April 1986, pages 483-490, Munich, DE; H. GLAESER et al.: "Biochemische Charakterisierung der in Molkereikefir und Kefirkulturen vorkommende Hefen" * Page 489, tables 6,III,7-9 * ---	1-7	
P,Y	PATENT ABSTRACTS OF JAPAN, vol. 13, no. 236 (C-602)[3584], 30th May 1989; & JP-A-1 47 338 (YAKULT HONSHA CO., LTD) 21-02-1989 * Abstract * -----	1-7	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 23 C
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 15-05-1990	Examiner DESMEDT G.R.A.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document			

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